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Stability and Sterility of a Recombinant Factor VIII Concentrate Prepared for Continuous Infusion Administration

Asim F. Belgaumi,[1] Christian C. Patrick,[2,3,4] and Steven R. Deitcher[5,6]*

[1]Department of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee
[2]Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee
[3]Pathology and Laboratory Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee
[4]Department of Pediatrics, The University of Tennessee, Memphis, Tennessee
[5]Department of Medicine, The University of Tennessee, Memphis, Tennessee
[6]Special Coagulation Laboratory, The University of Tennessee, Memphis, Tennessee

Minipumps may facilitate cost-effective and convenient continuous infusion (CI) therapy for severe hemophilia A. This study evaluated the in vitro sterility, ability to support bacterial growth, and specific activity stability of a recombinant factor VIII (FVIII; Bioclate™, Centeon) delivered by simulated CI at a variety of temperatures and after the addition of heparin or antibiotic. Closed system CIs of Bioclate™ (89.5 IU/ml) with and without heparin were sampled and cultured over a 6 day period. Bioclate™ (53.7 IU/ml) with and without heparin or vancomycin was inoculated with 102-105 CFU/ml of *S. aureus, S. epidermidis, Escherichia coli, E. cloacae,* or *Y. enterocolitica* and assessed by quantitative culture after 1 and 3 days. The stability of Bioclate™ (50, 100, and 250 IU/ml) at three temperatures (21°C, 37°C, and 39°C) with and without heparin or vancomycin was tested over a period of 28 days. FVIII activity was measured in triplicate by a chromogenic assay (Coamatic® Factor VIII, Chromogenix) and purity evaluated by Western blot. No bacterial growth was detected during CI of FVIII for up to 6 days. Following bacterial inoculation, there was rapid growth (>3 log increase) of all tested bacterial species except *S. aureus* which only displayed a 1 log expansion at 3 days. The addition of heparin containing 9.45 µg/U benzyl alcohol had no effect on bacterial growth. The addition of vancomycin caused a modest suppression of *S. aureus* growth but not of *E. coli.* Diluent alone did not support bacterial growth. Neither concentration, increased temperature, nor the addition of heparin or vancomycin had a significant effect on FVIII activity stability. Samples retained >75% baseline activity for between 3 and 7 days, except the infusion of Bioclate™ 50 IU/ml plus heparin maintained at 21°C which remained stable for 28 days. Western blot analysis supported the activity assay findings. Standard and concentrated preparations of Bioclate™ are suitable for CI when delivered by the MiniMed® 404-SP minipump. Because of the observed nutritive capability of this FVIII concentrate for sustaining bacterial growth, any contamination could result in systemic infection. Am. J. Hematol. 62:13–18, 1999. © 1999 Wiley-Liss, Inc.

Key words: hemophilia; prophylaxis; factor VIII; minipumps; continuous infusion

INTRODUCTION

Intravenous, bolus replacement therapy with factor VIII (FVIII) concentrates remains the mainstay of acute hemorrhage management and prophylactic therapy in patients with hemophilia A. Over the past few years, however, there has been an increase in the use and acceptance of continuous infusion (CI) as a FVIII delivery method, especially in patients following trauma or for perioperative administration. The CI of coagulation factors, in the form of cryoprecipitate and glycine precipitated plasma,

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*Correspondence to: Steven R. Deitcher, Department of Vascular Medicine, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk S-60, Cleveland, OH 44195. E-mail: deitchs@cesmtp.ccf.org

Dr. Belgaumi's current address is: P.O. Box 3354, MBC 64, Department of Oncology, King Faisal Specialist Hospital & Research Centre, Riyadh 11211, Saudi Arabia.

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was reported as early as 1970 [1]. More recent reports using highly purified FVIII concentrates have confirmed the utility, efficacy, and convenience of CI FVIII concentrate delivered by minipumps for patients with hemophilia A [2–5]. CI of highly concentrated preparations of recombinant FVIII via implantable pumps is being intensely investigated.

The use of more prolonged infusions of coagulation protein concentrates raises questions related to maintenance of procoagulant activity and sterility. Early studies of CI reported the use of FVIII diluted in large volumes (e.g., 500 ml of normal saline) [3,4]. Such a significant dilution of the FVIII protein and any accompanying stabilizers, can greatly limit the structural stability and specific activity of the concentrate. Dilution, itself, may lead to an immediate loss of 20–40% of expected FVIII recovery possibly due to adsorption of the unstable factor to the container wall [6]. The development of infusion minipumps, which can infuse at constant and extremely low rates, has facilitated CI of FVIII concentrates in standard post-reconstitution volumes, and possibly in highly concentrated forms. Recent studies have shown that the activity of standard dilution, monoclonal antibody purified, human plasma-derived FVIII concentrate can be maintained at room temperature for periods longer than the manufacturer's suggested 1 to 3 hr, allowing the use of these products for continuous infusion therapy [4,7,8].

A second concern with prescribing prolonged infusions of FVIII concentrates is the potential risk for bacterial contamination of the infusate and the subsequent development of bacterial sepsis in the patient. Such complications have been reported with the infusion of other products such as albumin, plasma and other blood products [9,10]. These products, like FVIII concentrates, are rich in protein and may support bacterial growth. The limited bacteriological studies of FVIII which have been reported to date, though, have failed to show any significant promotion of bacterial growth by the FVIII concentrate used [8].

In the present study we evaluated the stability and sterility of a readily available first-generation recombinant FVIII concentrate, Bioclate™ (manufactured by Baxter-Hyland, Hayward, CA and distributed by Centeon, King of Prussia, PA) prepared for simulated CI administration. This study specifically addressed the effect of post-reconstitution FVIII concentration, temperature, and the presence of additives such as antibiotics or heparin on specific activity stability for up to 1 month. We also conducted bacteriological studies on Bioclate™, in a simulated CI setting, to determine the potential for bacterial contamination and support of bacterial growth by the reconstituted factor in the presence and absence of an antibiotic or preservative-containing heparin.

MATERIALS AND METHODS

Factor VIII Concentrate

The FVIII product used throughout this study was Bioclate™ (manufactured by Baxter-Hyland, Hayward, CA and distributed by Centeon, King of Prussia, PA). In all experiments, the product was reconstituted with the sterile water provided by the manufacturer. All vials of Bioclate™ used in this study contained 537 IU of FVIII and were derived from a single lot. Various final concentrations of the FVIII were produced by simply altering the volume of sterile water used during reconstitution. Final concentrations between 50 IU/ml and 250 IU/ml were used for different experiments in this study.

Stability Studies

The stability of Bioclate™ (50, 100, and 250 IU/ml final concentrations) at 3 distinct temperatures (21°C, 37°C, and 39°C) with and without added porcine heparin sodium (SoloPak Laboratories, Elk Grove Village, IL) or vancomycin (Vancocin® HCl, Eli Lilly and Company, Indianapolis, IN) was tested for a period of up to 28 days. Heparin was added to FVIII concentrate in order to achieve a final concentration of 5 U/ml; the vancomycin was added to achieve a final concentration of 25 μg/ml. Each specially prepared FVIII specimen was divided between six 3 ml syringe reservoirs (Model No. MMT-103, MiniMed® Technologies, Sylmar, CA) designed for use in the MiniMed® Model No. 404-SP infusion minipump. Concentrate containing syringes, without attached tubing, were maintained in an incubater set to one of the specified temperatures. One syringe from each group was removed for testing at baseline and after 1, 3, 7, 14, and 28 days of incubation. Concentrates were not continuously pumped during incubation because of a limited supply of minipumps to support these experiments and only one sample of each type was evaluated to derive data. FVIII functional activity was measured in triplicate using a chromogenic assay (Coamatic® Factor VIII, Chromogenix AB, Mölndal, Sweden). FVIII integrity was evaluated by Western blot analysis by using a sheep anti-human factor VIII:C IgG peroxidase conjugate (SAF8C-HRP, Enzyme Research Laboratories Inc., South Bend, IN) and computer aided band density measurement on samples taken 0, 1, 3, 7, 14, and 28 days after the start of each experiment [11]. Results of the stability studies are expressed as the log percent residual FVIII activity versus time.

Maintenance of Sterility

Reconstituted FVIII (89.5 IU/ml) was drawn into two sterile 3 ml syringes (Model No. MMT-103, MiniMed® Technologies) which were individually inserted into two MiniMed® 404-SP infusion minipumps and connected to sterile extension tubing (Polyfin™ Extension Set, Model

No. MMT-128, MiniMed® Technologies). The tubing was primed with FVIII concentrate and connected, by using a 23G hypodermic needle, to a sterile evacuated blood collection tube (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) whose vacuum had been equilibrated with atmospheric pressure. All connections were made by using sterile precautions and techniques customarily employed during patient care, including scrubbing all surfaces with povidine-iodine solution and alcohol. The infusion pumps were set to deliver FVIII into the collection tubes at a rate of 0.02 ml/hr. The pumps, tubing and the collection tubes were kept at room temperature for the duration of the experiment. Laminar flow-equiped laboratory culture hoods were not used during these experiments. Samples (100 μl) of FVIII concentrate were collected for quantitative culture on sheep blood agar at baseline and after 3 and 6 days of simulated CI. Samples were allowed to drip directly onto the sheep blood agar plates and were incubated for 24 hr at 37°C. Similar experiments, in duplicate, were conducted using FVIII concentrate containing preservative free porcine heparin sodium (Lyphomed™, Fujisawa USA Inc., Deerfield, IL) at a final concentration of 10 U/ml.

Bacteriological Studies

In order to test the capacity of Bioclate™ to function as a bacterial growth medium, reconstituted FVIII was inoculated with escalating concentrations of several bacterial strains and assessed by quantitative culture. Individual aliquots of FVIII (53.7 IU/ml), housed in sterile MiniMed® syringes, were inoculated with 10^2, 10^3, 10^4, or 10^5 CFU/ml of *Staphylococcus aureus* ATCC 10832 or ATCC 8096, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidus* ATCC 35984, *Enterobacter cloacae* ATCC 35030, or *Yersinia enterocolitica* ATCC 27729. These bacteria were chosen because of their ability to cause infection due to environmental contamination or their ability to contaminate blood products. Each bacterial species was individually tested in duplicate at each concentration. The syringes were maintained at room temperature in order to simulate a condition expected during CI prophylaxis. A sample from each FVIII aliquot was removed and plated on sheep blood agar at baseline and 1 and 3 days after bacterial inoculation. The sheep blood agar plates were incubated at 37°C for 24 hr after which bacterial colony counts were performed according to standard methods [12]. Similar experiments that used *S. aureus* ATCC 10832 and *E. coli* ATCC 25922 as the inoculating bacterial strains were conducted on FVIII diluent alone, FVIII containing 25 μg/ml of vancomycin, and FVIII containing 5 IU/ml of preservative-containing porcine heparin sodium (SoloPak).

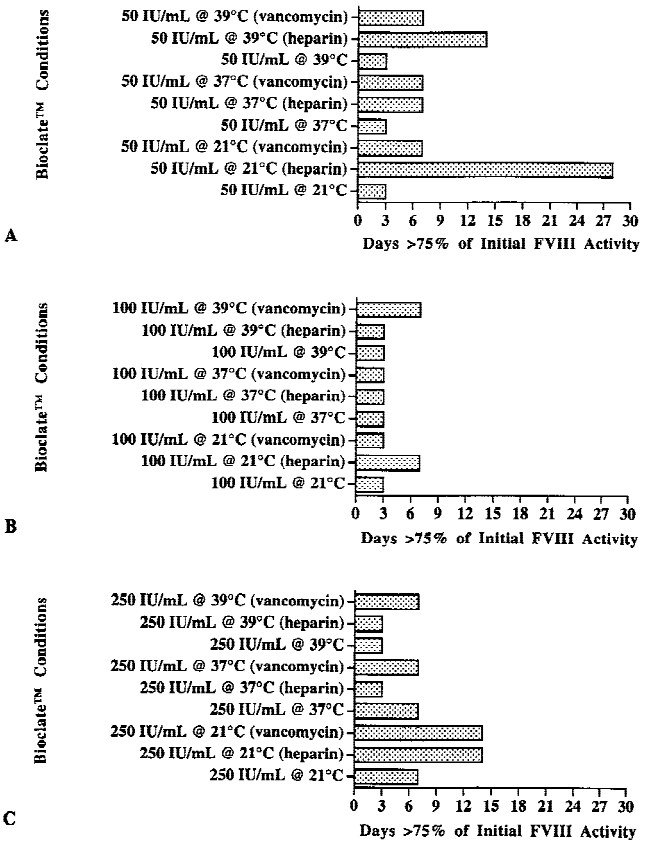

Fig. 1. Stability of reconstituted and stored FVIII concentrate (Bioclate™) at different concentrations, different storage temperatures, and with or without added heparin or vancomycin. A: Bioclate™ reconstituted to 50 IU/mL. B: Bioclate™ reconstituted to 100 IU/mL. C: Bioclate™ reconstituted to 250 IU/mL.

RESULTS

Specific Activity Stability

Neither FVIII starting concentration, storage temperature, nor the addition of heparin sodium or vancomycin had a consistently significant effect on FVIII specific activity stability (Fig. 1). Most samples retained >75% of baseline specific activity for between 3 and 7 days. No sample retained >75% of baseline specific activity for less than 3 days, whereas several retained this degree of activity for between 14 and 28 days. The sample of Bioclate™ 50 IU/ml containing heparin and maintained at 21°C maintained stability at 28 days. Western blot analysis supported the activity assay findings by demonstrating that factor degradation and not adsorption to plastic tubing was responsible for the gradual decrease in residual activity with time (data not shown).

Maintenance of Sterility

No bacterial growth was detected during the 6 day-long simulated CI of FVIII concentrate. Sterility was maintained despite brief periods of infusion discontinu-

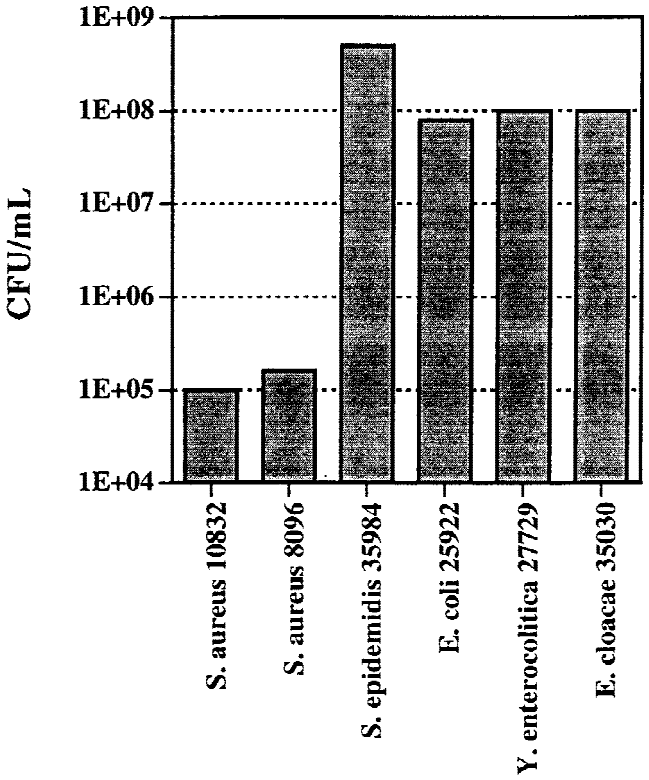

Fig. 2. Bacterial colony counts on incubation (21°C) day 3 of Bioclate™ (53.7 IU/mL) originally inoculated with 10^4 CFU/mL of different bacterial species. (y-axis values presented using scientific notation where, for example, 1E + 04 equals 10,000.)

ation and disconnection of the closed system for the purpose of sample collection.

Bacterial Growth

Following bacterial inoculation, there was rapid and significant growth (> 3 log CFU/ml increase in colony count) of all tested bacterial species at all dilutions except *S. aureus* which only displayed a 1 log expansion at 3 days following a 25,000 CFU/ml inoculum (Fig. 2). This limited growth of *S. aureus* was not strain specific. Bacterial growth rate was independant of initial inoculum for all species. The addition of porcine heparin sodium (5 U/ml) containing benzyl alcohol (9.45 μg/U) as a preservative had no significant effect on bacterial growth in this system. The addition of vancomycin (25 μg/ml) caused a modest suppression of *S. aureus* growth but did not affect the growth of *E. coli* (Fig. 3). FVIII diluent alone did not support growth of the tested microorganisms, but led to a progressive decline in colony concentration with time confirming the non-nutritive nature of this fluid (data not shown).

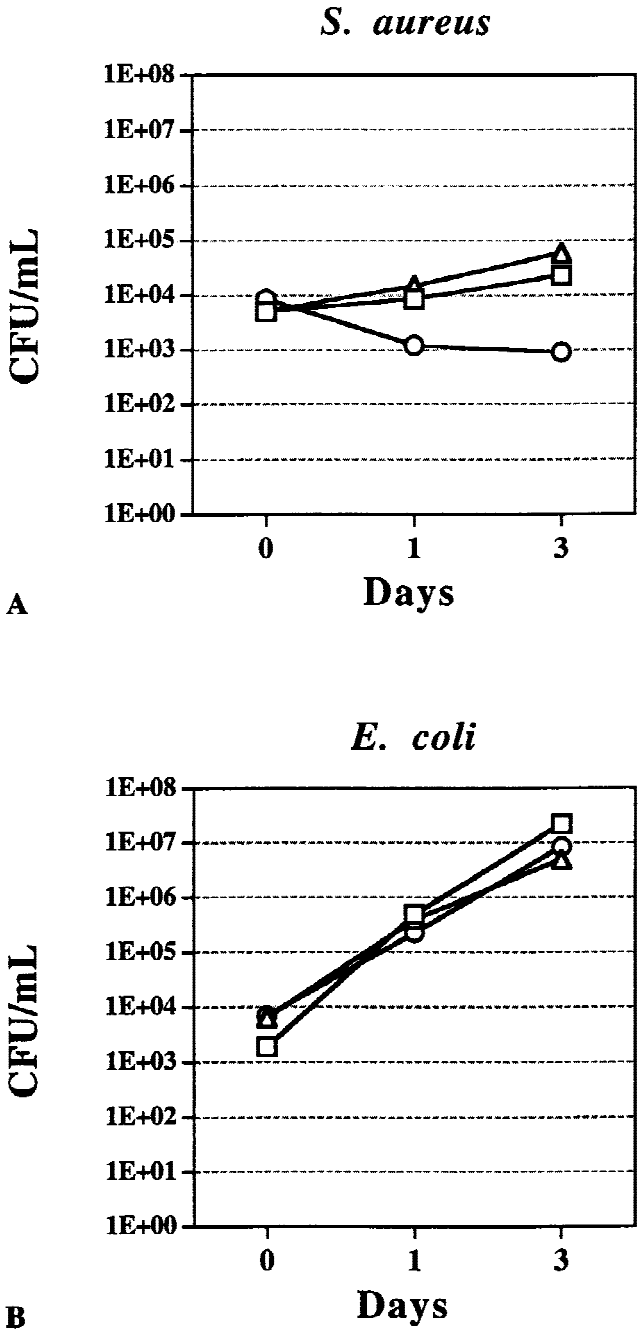

Fig. 3. Effect of porcine sodium heparin (5 U/mL final concentration) containing benzyl alcohol (9.45 μg/U) and vancomycin (25 μg/mL final concentration) on the growth of *S. aureus* ATCC 10832 (A) and *E. coli* ATCC 25922 (B) in Bioclate™ (53.7 IU/mL). (y-axis values presented using scientific notation where, for example, 1E + 04 equals 10,000). ❐ = Bioclate™ without additive. △ = Bioclate™ with heparin. ○ = Bioclate™ with vancomycin.

DISCUSSION

This study demonstrates that, when prepared for ambulatory CI by using the MiniMed® 404-SP infusion pump, syringe reservoir, and polyolefin-lined Polyfin™ extension set, the recombinant FVIII concentrate, Bioclate™, retains greater than 75% of procoagulant activity for a minimum of 3 days. These data support the use of Bioclate™ in concentrations up to 250 IU/ml for ambu-

latory CI administration as long as the reservoir of FVIII is changed at least every 3 days. Our findings are in agreement with the reports of others who demonstrated that the FVIII activity of commercial concentrates remains stable well beyond the 3 hr suggested in manufacturer package inserts [4,5,7,8]. Unlike the reports of others, our investigations into FVIII specific activity stability were conducted at temperatures relevant to clinical care; namely, room temperature (21°C) where a minipump would reside, body temperature (37°C) which would be the temperature of the FVIII infusing through an indwelling catheter, and 39°C to simulate a febrile patient. It is important to characterize any FVIII concentrate selected for CI administration under conditions that reflect various real-life situations.

Although there was a steady decrease in FVIII specific activity over time at all temperatures studied, greater than 75% of activity was retained for between 3 and 7 days following reconstitution. Descrepancies in the data, where some samples retained greater than 75% of baseline activity for significantly longer durations than other samples, may be due to the specific nature of a samples FVIII concentration, storage temperature, and added drug. Western blot analysis demonstrated stable quantities of total protein but decreasing amounts of intact FVIII as time progressed. This finding suggests progressive protein degradation as the cause of progressive activity loss and not adsorption to the syringe reservoir or polyolefin-lined tubing.

While the use of prophylactic infusions of FVIII concentrates to prevent spontaneous bleeding in Hemophilia A patients is not a novel concept, the use of CI of FVIII for prophylaxis is relatively untested [13–15]. The use of minipumps and implantable pumps to deliver ambulatory CI of FVIII concentrates may facilitate cost-effective and convenient long-term prophylactic therapy for severe hemophilia A patients. Advantages of CI prophylaxis compared with bolus regimens include the ease of dose adjustment based on random activity measurements, maintenance of a constant level of FVIII activity, less cumulative factor exposure, and potential cost savings [16,17]. Challenges to effective CI prophylaxis include the infectious and thrombotic risks of long-term vascular access, alterations in body self-image, uncertain efficacy compared to bolus prophylaxis regimens with associated high peak factor activity levels, and factor concentrate stability and sterility. FVIII concentrate stability has been shown to be product and infusion device specific and greatly reduced by dilution [5,6].

Our stability experiments were conducted by using a commercially available FVIII concentrate which was reconstituted to three different final concentrations of FVIII protein (50 IU/ml, 100 IU/ml, and 250 IU/ml) by using diluent provided by the manufacturer. In order to accommodate the use of ultracompact pumps and infrequent reservoir refilling, CI prophylaxis will require the use of concentrated FVIII preparations. FVIII concentrate reconstituted in the manner used in this study (up to 250 IU/ml), would result in a hypertonic solution that would provide a very high concentration of FVIII activity at any catheter tip, blood stream interface. These characteristics of highly concentrated factors could precipitate a catheter-associated venous thrombosis; thus, the addition of a small amount of heparin to the concentrate seems prudent [18]. Our study demonstrated that the addition of heparin (5 U/ml) had no adverse effect on FVIII specific activity stability at any of the tested factor concentrations or incubation temperatures. Devices such as the MiniMed® 404-SP infusion minipump are easy to carry, conceal, operate, and cause minimal interference with a patient's daily activities. Pumps like this are gaining popularity for the CI insulin management of patients with diabetes and seem equally suitable for use in hemophilia [19].

The other major concern with CI therapy is the potential for bacterial contamination and overgrowth of the reconstituted FVIII, potentially resulting in line infection, bacteremia, sepsis, and the need for line removal. As long as aseptic techniques were used, we did not encounter bacterial contamination in any of our simulated infusions. However, we did document the considerable support of bacterial growth provided by Bioclate™. We have tested a variety of different bacterial species implicated in central venous access and blood product-related infections. There was rapid expansion of bacterial cultures for all the bacteria tested, except for *S. aureus*, where the growth was less precipitous. Whether the FVIII itself or added proteins, like albumin, provide the nutritive materials is unclear. Our results differ from those of Schulman et al., who previously reported that reconstituted plasma-derived FVIII did not support bacterial growth any more than control, crystalloid solutions [8]. They, however, only studied bacterial growth for a 24 hr period and at a single inoculum load. We have shown considerable bacterial growth over a 72 hr period of time regardless of inoculum size. This fact becomes highly relevant when one contemplates use of CI prophylaxis, where the factor reservoir might be changed every 3 days.

A recent report by Hardie et al. suggested the possibility that preservatives in heparin sodium may function as bacteriostatic agents when added to FVIII concentrates [20]. Our study failed to corroborate this finding by demonstrating no difference in bacterial growth of *S. aureus* and *E. coli* when incubated in FVIII prepared with and without added heparin sodium (10 U/ml) containing 9.45 mg/U of the preservative benzyl alcohol.

There continues to be controversy regarding the efficacy of using a vancomycin-containing flush solution for the prevention of indwelling central venous catheter in-

fection caused by sensitive Gram-positive organisms [21–23]. Addition of vancomycin to the reconstituted factor resulted in a reduction of *S. aureus* growth, as expected, but no such bacteriostatic activity was seen towards *E. coli.* We are in agreement with the recommendations of the Centers for Disease Control Subcommittee on Prevention and Control of Antimicrobial-Resistant Microorganisms in Hospitals, by not recommending the addition of prophylactic vancomycin to FVIII infusions because the benefit of retarding infection from a limited spectrum of potential contaminating organisms is negated by the more serious potential for suborning antibiotic resistance [24].

In summary, we have shown that Bioclate™ is suitable for ambulatory CI administration in severe hemophilia A patients when delivered by the MiniMed® 404-SP infusion pump, syringe reservoir, and polylefin-lined Polyfin™ extension set. Bioclate ™ retained 75% of its specific activity for at least 3 days at room temperature, normal body temperature, and under simulated febrile conditions. Although sterility can be maintained by employing standard aseptic techniques, the observed nutritive capability of this FVIII concentrate for sustaining the growth of a broad spectrum of bacterial organisms suggests that any contamination during CI prophylaxis could result in serious systemic infection.

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